

[0192] In an illustrative embodiment, the first thermoelectric cooling device **1144** is used to control the temperature of the waste well **180** region of the microfluidic device to a constant temperature of 25° C. The second thermoelectric cooling device **1142** is used to ramp the temperature of the analysis region **135** of the microfluidic device from 60° C. to 95° C., at a rate within the range of 0.1° C./s to 1.0° C./s inclusive, so that a stopped-flow type thermal melt analysis can be performed on amplicons present within the channels within the analysis region **135**.

[0193] One feature of the interface module **1100** that facilitates independent temperature control of the waste well and analysis regions of the microfluidic device is that the first **1140** and second **1145** thermal blocks are separated by an air gap, which inhibits heat transfer between the first and second thermal blocks. Although the two thermal blocks **1140**, **1145** are separated by an air gap, the two thermoelectric heaters **1142**, **1144** are both attached to a common heat sink **1150**. The use of a common heat sink **1150** provides benefits over the use of separate heat sinks in embodiments in which the temperature setpoint of the analysis region **135** is higher than the temperature setpoint of the waste well **180** region. These benefits arise because the heat is typically removed from the waste well portion of the microfluidic device in order to maintain that portion at a constant temperature of 25° C., while heat must be added to the analysis portion of the microfluidic device in order to reach the temperature required to perform thermal melt analysis. Since the heat sink **1150** removes heat from one thermoelectric cooler and adds heat to the other thermoelectric cooler, the temperature tends to stabilize at around 30° C. Since thermoelectric coolers work best when thermal gradients are minimized between the heated or cooled object and the heat sink, this improves efficiency of the overall system. When the system shown in FIGS. 11A-11D is operated, the temperature of the two regions of the microfluidic device can be independently controlled. Although the use of a common heat sink may be particularly advantageous, the use of separate heat sinks for different thermoelectric coolers is also compatible with embodiments of the invention.

[0194] The instrument shown in FIGS. 11A-11D and the microfluidic device shown in FIG. 10 were used to amplify an 85 bp target from genomic DNA in the presence of the DNA binding dye SYBR Green I, which is fluorescent when bound to double-stranded DNA. In the microfluidic device, the amplification products were subjected to a thermal gradient from 60° C. to 95° C. at a rate of approximately 0.1° C./s. FIG. 12A shows the thermal ramp (below) and the change in fluorescent signal (above) over time. The boxed area in the upper portion of FIG. 12A is the region of the DNA thermal melt. FIG. 12B plots the negative change in fluorescence (dF) divided by the change in temperature (dT) as a function of temperature, for the boxed region in FIG. 12A. The temperature at the single peak in this plot represents the temperature at the midpoint of the DNA denaturation curve, or the T<sub>m</sub> value for the 85 bp target.

#### [0195] Assay Kits

[0196] The present invention also provides kits for conducting the binding assays of the invention. In particular, these kits typically include microfluidic devices, systems, modules and workstations for performing the assays of the invention. A kit optionally contains additional components for the assembly and/or operation of a multimodule workstation of the invention including, but not restricted to robotic elements (e.g., a track robot, a robotic armature, or the like), plate handling devices, fluid handling devices, and computers (including e.g., input devices, monitors, CPU, and the like).

[0197] Generally, the microfluidic devices described herein are optionally packaged to include reagents for performing the device's functions. For example, the kits can optionally include any of the microfluidic devices described along with assay components, buffers, reagents, enzymes, serum proteins, receptors, sample materials, antibodies, substrates, control material, spacers, buffers, immiscible fluids, etc., for performing the assays of the invention. In the case of prepackaged reagents, the kits optionally include pre-measured or pre-dosed reagents that are ready to incorporate into the assay methods without measurement, e.g., pre-measured fluid aliquots, or preweighed or pre-measured solid reagents that can be easily reconstituted by the end-user of the kit.

[0198] Such kits also typically include appropriate instructions for using the devices and reagents, and in cases where reagents are not predisposed in the devices themselves, with appropriate instructions for introducing the reagents into the channels and/or chambers of the device. In this latter case, these kits optionally include special ancillary devices for introducing materials into the microfluidic systems, e.g., appropriately configured syringes/pumps, or the like (in one embodiment, the device itself comprises a pipettor element, such as an electropipettor for introducing material into channels and chambers within the device). In the former case, such kits typically include a microfluidic device with necessary reagents predisposed in the channels/chambers of the device. Generally, such reagents are provided in a stabilized form, so as to prevent degradation or other loss during prolonged storage, e.g., from leakage. A number of stabilizing processes are widely used for reagents that are to be stored, such as the inclusion of chemical stabilizers (i.e., enzymatic inhibitors, microbicides/bacteriostats, anticoagulants), the physical stabilization of the material, e.g., through immobilization on a solid support, entrapment in a matrix (i.e., a bead, a gel, etc.), lyophilization, or the like.

[0199] The elements of the kits of the present invention are typically packaged together in a single package or set of related packages. The package optionally includes written instructions for carrying out one or more target independent assay in accordance with the methods described herein. Kits also optionally include packaging materials or containers for holding microfluidic device, system or reagent elements.

[0200] The discussion above is generally applicable to the aspects and embodiments of the invention described herein. Moreover, modifications are optionally made to the methods and devices described herein without departing from the spirit and scope of the invention as claimed, and the invention is optionally put to a number of different uses including the following:

[0201] The use of a microfluidic system containing at least a first substrate and having a first channel and a second channel intersecting the first channel, at least one of the channels having at least one cross-sectional dimension in a range from 0.1 to 500  $\mu\text{m}$ , in order to test the effect of each of a plurality of test compounds on a biochemical system comprising one or more focused cells or particles.

[0202] The use of a microfluidic system as described herein, wherein a biochemical system flows through one of said channels substantially continuously, providing for, e.g., sequential testing of a plurality of test compounds.

[0203] The use of a microfluidic device as described herein to modulate reactions within microchannels.

[0204] The use of electrokinetic injection in a microfluidic device as described herein to modulate or achieve flow in the channels.